PATENT

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Bavykin, et al.
Application:	METHOD FOR LABELING DNA AND RNA
Serial No.:	10/057,753
Filing Date:	January 23, 2002
Art Unit:	1637
Examiner:	Kim, Young J
Case No.:	0003/00377

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first Class Mail, pursuant to 37 C.F.R. 1.8 to the Commissioner for Patents, Alexandria, VA 22313-1450 on

October 14, 2004 (Date of Deposit)

Jillian Szafranski — Jillian Szafranski — 10/14/2004

Name of Representative — Signature — Date of Signature

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Commissioner For Patents
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37 C.F.R. 1.131 Affidavit of
SERGEI G. BAVYKIN

Dear Sir:

Dr. Sergei G. Bavykin, being first duly sworn, deposes and says that:

1. I am a joint inventor of the invention described and claimed in the above-identified patent application.

2. I declare that conception of the invention occurred in the United States.

3. A method for labeling DNA and RNA as described and claimed in the above-mentioned patent application was reduced to practice in 1998. Attached is an invention report summary (Tab A) and an Invention Report (TAB B), signed by me on

October 14, 1998. Both the invention report summary and the Invention Report describe the invention as claimed in claims independent claims 1 and 9.* Specifically, the first paragraph of the summary and of the Invention Report describes how DNA and RNA is reacted with hydrogen peroxide and a coordination complex. These reports note the formation of free aldehyde forms of the ribose or deoxyribose result, particularly in anaerobic conditions.

4. Paragraph three of the summary discloses how the aldehyde moieties react with amine (ethylenediamine) to form a Schiff base (condensation reaction). Reduction of the Schiff base and labeling is also disclosed in this paragraph.

5. Under *Procedures* of the Invention Report, two different protocols of the claimed procedure are provided, including use of specific radical producing agents, and specific amines. Also in this section, the invention is taught occurring at temperatures below the boiling point of water, as recited in claim 16. Specifically, that portion of the Invention Report discloses a 45 ℃ reaction temperature. This section of the Invention Report also teaches the use of labels and the employment of anaerobic environments.

6. The "Description" paragraph of the Invention Disclosure Record and Evaluation (also attached) teaches all of the claimed elements of the invention, as discussed above. The employment of the various amines, and radical generating complexes in anaerobic environs is finally depicted in FIGS. 1 and 2 of the *Procedure* section of the Invention Report. FIG. 2 of the Report and FIG. 1A of the patent application depict virtually identical condensation and labeling reactions.

* These inventions were explained to invention evaluator Dr. Victoria Henson-Apollonio during 1998 and transcribed by her in the normal course of business at the technology transfer program at Argonne National Laboratory, Argonne, Illinois. This can be noted on the last page of the invention summary (Tab A).

7. The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of any resulting patent.

October 13, 2004
Date

Sergei Bavykin, Ph.D.

INVENTION REPORT

ANL CASE #: ANL-IN-98-093

DOE CASE #:

INVENTORS: Sergei G. Bavykin
Andrei Mirzabekov

TITLE: **A NEW METHOD FOR LABELING DNA AND RNA**

DESCRIPTION: This invention embodies a novel method for labeling DNA and RNA molecules with compounds containing primary amines. In this process, DNA or RNA is modified under anaerobic conditions by a reaction in which the nucleic acid is reacted with hydrogen peroxide (H_2O_2) and one of the coordination complexes such as 1,10-phenanthroline-Cu(II) (OP-Cu), bleomycin-Fe(III) (BLM-Fe), EDTA-Fe, ascorbic acid-Cu, methylene-blue-Cu, metallophorphyrins, and other "chemical nucleases" (compounds that introduce single-stranded breaks into double-stranded nucleic acids under aerobic conditions). Under anaerobic conditions, H_2O_2 and chemical nucleases produce free radicals that attack nucleic acids and initiate formation of free nucleic acid bases and the aldehyde form of ribose or deoxyribose. A reactive aldehyde group on the DNA or RNA results, and serves as the substrate for subsequent labeling reactions.

In one embodiment of this invention, ethylene diamine (EDA) is included in the initial reaction mixture in the presence of H_2O_2 and OP-Cu or EDTA-Fe. The EDA reacts with the aldehyde groups of the DNA or RNA to form a Schiff base (EDA=nucleic acid). The labeling process is continued by reducing this double bond with a reducing agent such as sodium cyanoborohydride. The product of this reduction is then labeled by adding Texas Red sulfonyl chloride (TexRed-SuCl) and hybridized with a microchip.

In another embodiment of this invention, nucleic acids (both DNA and RNA) were modified with OP-Cu or EDTA-Fe in the presence of H_2O_2 and amino-dye Lissamine rhodamine B ethylenediamine (LissRH-EDA). The resulting LissRH-EDA=nucleic acid Schiff base was reduced with sodium cyanoborohydride. Alternatively, crosslinking of the dye and Schiff base reduction were integrated in the one step. Labeled nucleic acids were successfully hybridized with a microchip.

This invention provides for labeling under conditions that are simple, fast, and relatively mild, that result in high yields of crosslinked complexes. The method is only slightly dependent on the nucleic acid sequence or reaction temperature and it is possible to label both DNA and RNA using the same protocol.

Experiments have been carried out in which RNA and DNA were modified with H_2O_2 and OP-Cu or with H_2O_2 and EDTA-Fe and labeled with TexRed-SuCl or LissRH-EDA. RNA and DNA labeled using the inventive process were shown to be effective probes in hybridization experiments.

BACKGROUND, INCLUDING RELATED ART: See attached report written by Sergei Bavykin.

PUBLICATIONS, REPORTS AND TALKS: A manuscript is in preparation.

INVENTORS' STATUS: The inventors, Sergei Bavykin and Andrei Mirzabekov are employed by Argonne National Laboratory in the Center for Mechanistic and Biotechnology Division and are citizens of Russia. This invention was conceived under Contract No. W-31-109-ENG-38 between the U.S. Department of Energy (DOE) and The University of Chicago representing Argonne National Laboratory.

BADGE #'s: 49615 Sergei G. Bavykin; 47595 Andrei Mirzabekov

FUNDING SOURCE for research under which invention was conceived:

ANL: ___ LDRD (Laboratory Director Research & Development, previously called Exploratory Research Funds [ERF] or Program Development Funds [PDF])

Were LDRD (or ERF/PDF) funds used to support research that preceded the research during which the invention was conceived? _X_ No ____ Yes

DOE: FWP No. 8C346 B&R Code: 40-04

Non-ANL/DOE Sponsor: Name of organization: DARPA

Type of organization: _X_ Federal ____ State ____ Private ____ Not-for-profit

Type of funding document or agreement: ___ WFO ___ CRADA ____ HTSCA _X_ MIPR

Other (specify): _____ Agreement No.: MIPR No. 98-0236

PROBABLE VALUE: This invention is a new method of the SHOM related technologies.

RECOMMENDATIONS: The recommendations of ITD personnel will be provided later.

EXCEPTIONAL CIRCUMSTANCES: This invention is not an exceptional circumstance invention.

REPORT DATED: September 14, 1998 - Victoria Henson-Apollonio

READ AND UNDERSTOOD BY:

[signature]	10/14/98		
Inventor Signature	Date	Witness Signature	Date
Inventor Signature	Date	Witness Signature	Date

ANL-IN-98-09

May 27, 1998

INVENTION REPORT

INVENTORS: Sergei G. Bavykin, and Andrei D. Mirzabekov

TITLE: **New Method of DNA and RNA labeling with radical-producing chemical agents**

DESCRIPTION: Chemical radical-producing agents such as 1,10-phenanthroline-Cu(II) (OP-Cu) and bleomycin-Fe(III) (BLM-Fe) complexes are commonly used as "chemical nucleases" to introduce single-stranded breaks in nucleic acids. We explore the possibility that chemical radical-producing complexes such as 1,10-phenanthroline-Cu(II) (OP-Cu), bleomycin-Fe(III) (BLM-Fe), EDTA-Fe, ascorbic acid-Cu, methylen blue-Cu, metalporfirins and some other, called "chemical nucleases" because of their ability to cleave DNA under aerobic conditions (1-3), are able to generate amine/hydrazide-nucleic acid crosslinking under anaerobic conditions. This suggestion is based on the chemistry of BLM-Fe-mediated DNA modification (Fig.1). In the presence of hydrogen peroxide under anaerobic conditions the BLM-Fe complex catalyzes the formation of free nucleic acid bases and aldehyde form of deoxyribose at abasic site of DNA backbone, which undergoes scission only in the presence of alkali or amines (1). Similar reaction was proposed for the OP-Cu complex under the same conditions (1,2). We have suggested that the aldehyde form of ribose or deoxyribose at abasic site may react with amines, hydrazines, or hydrazides (Fig. 2) in similar way like it was described for fluorescent labeling of partially depurinated DNA with hydrazines earlier(4).

We have already developed two different protocols. Both of them we successfully used for *B. medusa* RNA labeling and fragmentation at the same time. We also demonstrate hybridization of the labeled RNA with Microbial Testing Microchip (Fig.3).

Procedures:

According *first* protocol RNA was modified with OP-Cu/H_2O_2 system in the presence of ethylenediamine (EDA) during 30 min at 45^{0}C (to provide anaerobic conditions all solutions just before start of reaction were briefly bubbled with argon). Obtained EDA=RNA Schiff base was reduced with sodium cyanoborohydride in the same tube for 30 min at room temperature, repricipitated with acetone, labeled with Texas Red sulfonyl chloride and hybridized with microchip.

According *second* protocol RNA was modified with OP-Cu/H_2O_2 system in the presence of amino-dye Lissamine rhodamine B ethylenediamine (LissRH-EDA) during 30 min at 45^{0}C in anaerobic conditions (argon bubbling before reaction starting). Obtained LissRH-EDA=RNA Schiff base was reduced with sodium cyanoborohydrride in the same tube for 30 min at room temperature and was hybridized with microchip.

Note: Both step in protocol *two* probably may be integrated in one.

The obvious advantages of fluorescent dye labeling with OP-Cu/H_2O_2: Simplicity, mild conditions of reaction, high yield of crosslinked complexes, short

incubation times, only slight dependence from DNA/RNA sequence, the feasibility to label both DNA and RNA according the same protocol. Moreover rate of reaction is only slightly dependent of the temperature.

References:

1. Stubbe, J. And Kozarich, J.W. (1987) *Chem. Rev. 87*, 1107-1136.
2. Sigman, D.S. (1990) *Biochemistry 29*, 9097-9105.
3. Sigman, D.S., Mazumder, A. and Perrin, D.M. (1993) *Chem. Rev.* 93, 2295-2316
4. Proudnikov, D.P., and Mirzabekov, A.D. (1996) *Nucl. Acids Res.* **24**, 4535-4532

Legends to Figures:

Fig.1. Aerobic and unaerobic way of DNA modification with radicals generated by bleomycin-Fe complex.

Fig.2. Partially depurinated DNA-labeling with TMR-hydrazine (NH_2NH-TMR) and fluorescein (FITC).

Fig.3 Hybridization of *B. medusa* RNA labeled by Texas Red (A) and LissRH-EDA (B) with Microbial Testing Microchip.

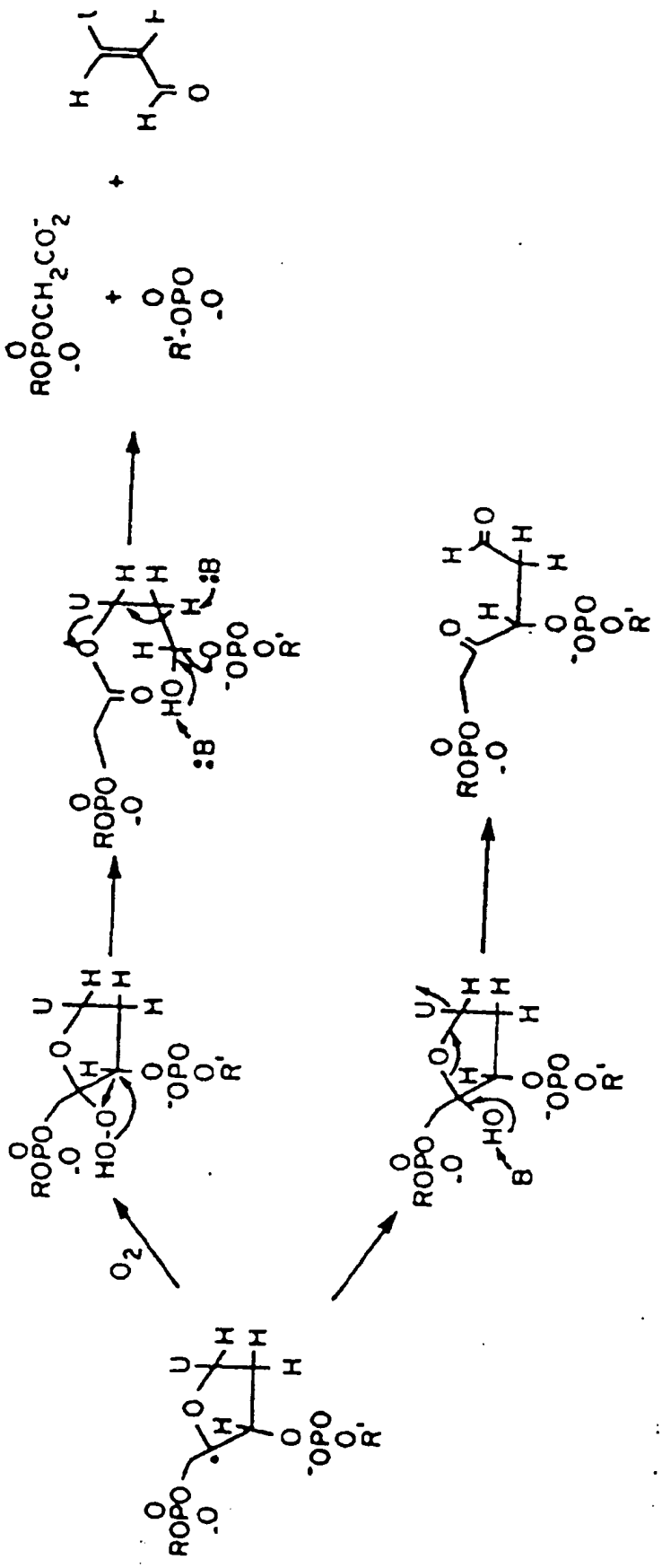

Fig. 1

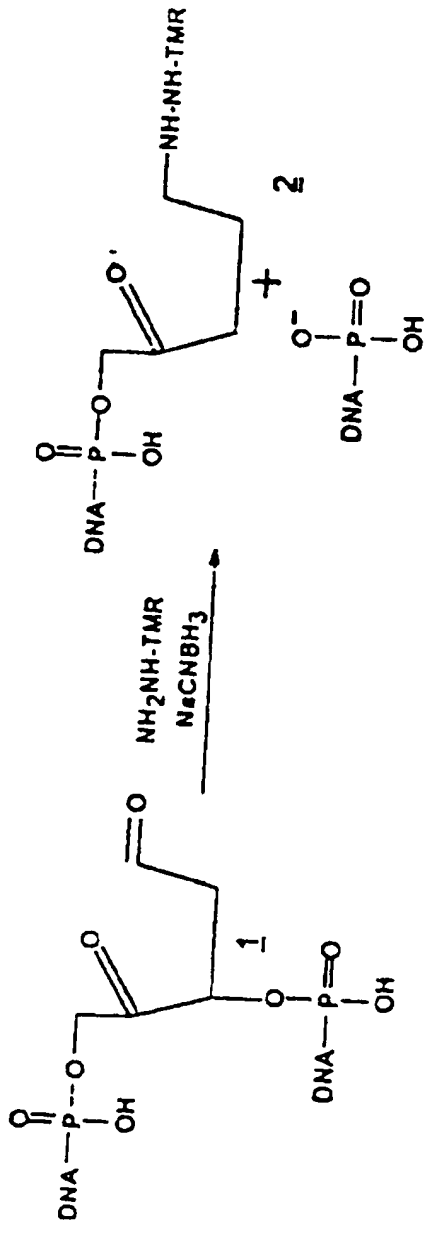

Fig. 2

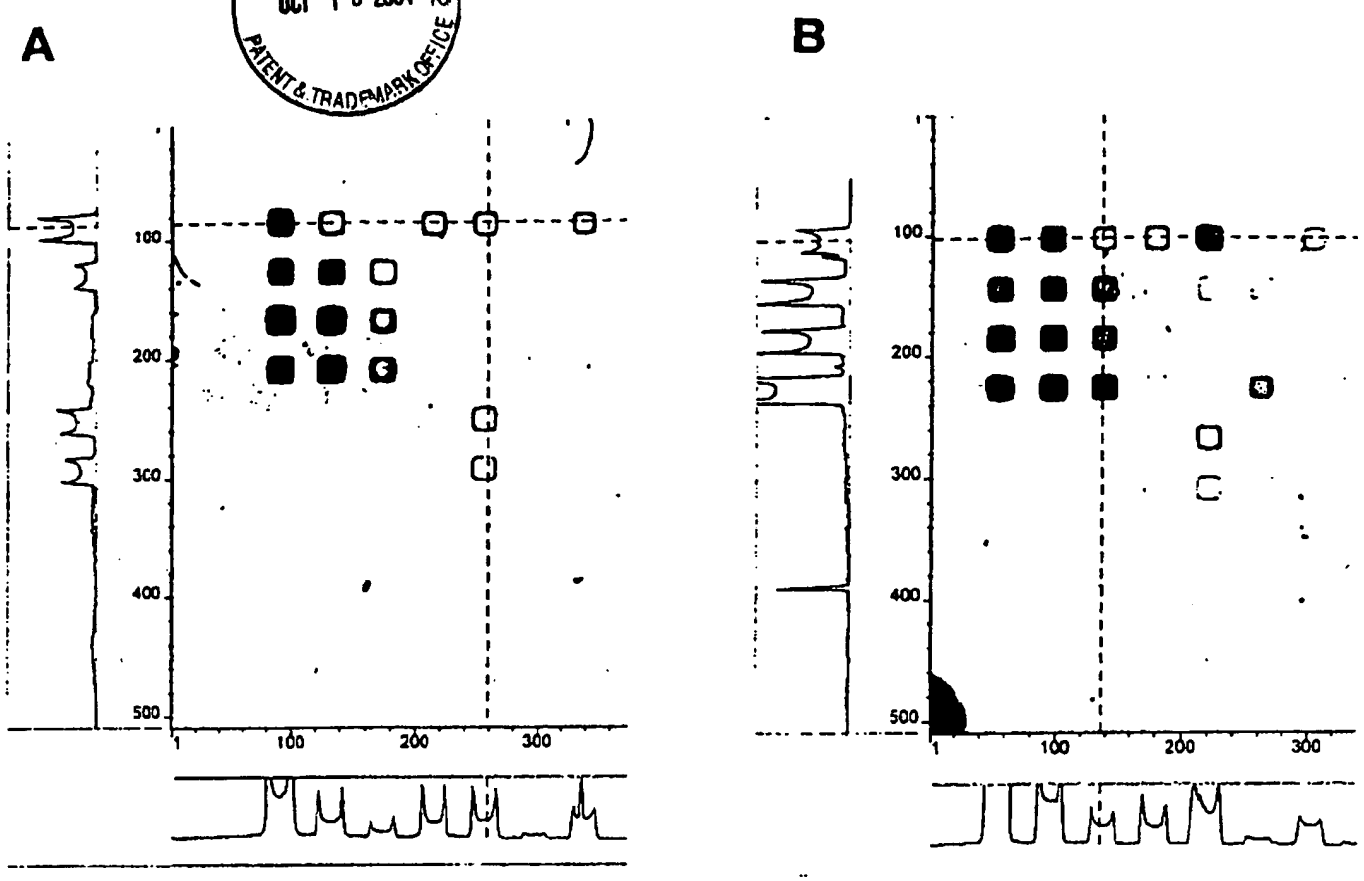

Fig. 3

INVENTION DISCLOSURE RECORD AND EVALUATION

ANL Case No. ANL-IN-98-093

The invention report appended hereto will serve to describe formally the invention.

1. Title of Invention: **A NEW METHOD OF LABELING DNA AND RNA**

2. Inventors (please print or type):

Full Name:	Sergei G. Bavykin
Residence Address:	16W747 Mockingbird Ln., Apt #106
City/State/Zip:	Hinsdale, IL 60521
County:	Dupage
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Full Name:	Andrei D. Mirzabekov
Residence Address:	1005 Village Court
City/State/Zip:	Darien, IL 60559
County:	Dupage
Work Phone:	(630)252-3161
Home Phone:	(630)241-9163

3. Signature of Inventor and Date:	Signature of Witness and Date:
10/15/98	

(Witnesses are attesting they understand the description of the invention and that the signatures of the inventors are valid.)

4. Brief Description of Invention Emphasizing Unique and Novel Aspects (attach separate sheet if necessary):

DESCRIPTION: This invention embodies a novel method for labeling DNA and RNA molecules with compounds containing primary amines. In this process, DNA or RNA is modified under anaerobic conditions by a reaction in which the nucleic acid is reacted with hydrogen peroxide (H_2O_2) and one of the coordination complexes such as 1,10-phenanthroline-Cu(II) (OP-Cu), bleomycin-Fe(III) (BLM-Fe), EDTA-Fe, ascorbic acid-Cu, methylene-blue-Cu, metallophorphyrins, and other "chemical nucleases" (compounds that introduce single-stranded breaks into double-stranded nucleic acids under aerobic conditions). Under anaerobic conditions, H_2O_2 and chemical nucleases produce free radicals that attach nucleic acids and initiate formation of free nucleic acid bases and the aldehyde form of ribose or deoxyribose. A reactive aldehyde group on the DNA or RNA results, and serves as the substrate for subsequent labeling reactions. This method as well as principle of labeling was not described before anywhere.

5. Dates and Places of Inventions: 5/27/98, ANL

Conception by Inventor		At:	
First Sketch or Drawing:	At:	In Workbook:	Page:
First Written Description:	At:	In Workbook:	Page:
Disclosure to Others: 10/97, Disclosed in discussion with Andrei Mirzabekov			
DARPA - Annual Report (Seminar)		At: 5/5/98	
		At:	
		At:	
Completion of Model or Full Size Device:		At:	
First Test or Operation of Invention: 3/5/98		At:	

6. Has your invention been reduced to practice? YES. If yes, what was the performance of the invention?

Laboratory test was run at March 5, 1998, Workbook p. 92-a.

If no, what further development work is needed to reduce the invention to practice and who will fund it? (Include an estimate of the cost and manpower that will be needed.)

7. What are the potential uses of this invention by the government and/or by industry? What needs does it fulfill? What advantages does it have over existing products or processes?

This method has been developed as biological microchip related technology.

8. List any industry contacts who have shown an interest in this invention or this line of work. Have you discussed the possibility of collaborative development through a CRADA? Did you use a non-disclosure agreement?

Motorola

9. Have you disclosed this invention and/or what plans do you have to disclose aspects of this invention - publications, talks, public use? Please give the dates.

The article describing this method is in preparation now.

10. List other R&D organizations working in the technology area of your invention, including key names where possible.

Affymetrix, HySeq Inc., NIH, Stanford, CIS BioInternational (France), Oxford (UK)

11. Do you recommend foreign filing of this invention? If yes, what countries?

Russia, UK, France, Germany

12. List key words that may be useful in searching for prior art or competing technologies:

Biological microchip, coordination complexes, 1,10-phenanthroline-Cu (II)
EDTA-Fe (III), H_2O_2